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**From:** Basi, Nirmal
**Sent:** Thursday, November 06, 2003 11:16 AM
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**Subject:** Sequence search US Application 09/430,775

Sequence search -
App. #: 09/430,775
Result format: Paper.
Title:Method of Obtaining compositions comprising Y4 specific compounds
Inventors:Bard et al
Priority Date:1/13/97

Please search:
i) SEQ ID NO:1, 2, 27, 28, 32 and 33 (SEQ ID NOs:2, 28, 32 and 33 are protein, 1 and 27 are nucleic acid)

Search commercial and issued database.

Thanks,
Nirmal S. Basi

Nirmal S. Basi
Art Unit 1646
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L1: Entry 1 of 1 File: USPT Nov 2, 1999

US-PAT-NO: 5976814
DOCUMENT-IDENTIFIER: US 5976814 A

TITLE: DNA encoding a human neuropeptide Y/peptide YY/pancreatic polypeptide receptor (Y4) and uses thereof

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Bard; Jonathan A. | Wyckoff | NJ | | |
| Walker; Mary W. | Elmwood Park | NJ | | |
| Branchek; Theresa | Teaneck | NJ | | |
| Weinshank; Richard L. | Teaneck | NJ | | |

US-CL-CURRENT: 435/7.2; 435/252.3, 435/320.1, 435/325, 435/69.1, 530/300, 530/350

CLAIMS:

What is claimed is:

1. A process for determining whether a chemical compound specifically binds to and activates a human or a rat Y4 receptor, which comprises contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a human or a rat Y4 receptor, with the chemical compound under conditions suitable for activation of the human or the rat Y4 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the human or the rat Y4 receptor, wherein the human Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 2 and the rat Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 28.

2. A process for determining whether a chemical compound specifically binds to and activates a human or rat Y4 receptor, which comprises contacting a membrane fraction from nonneuronal cells producing a second messenger response and expressing on their cell surface a human or rat Y4 receptor, with the chemical compound under conditions suitable for activation of the human or rat Y4 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the human or rat Y4 receptor, wherein the human Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 2 and the rat Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 28.

3. A process for determining whether a chemical compound specifically binds to and inhibits activation of a human or rat Y4 receptor, which comprises separately contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a human or rat Y4 receptor, with both the chemical compound and a second chemical compound known to activate the human or rat Y4 receptor, and with only the second chemical compound, under conditions suitable for

activation of the human or rat Y4 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the human or rat Y4 receptor, wherein the human Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 2 and the rat Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 28.

4. A process for determining whether a chemical compound specifically binds to and inhibits activation of a human or rat Y4 receptor, which comprises separately contacting a membrane fraction from nonneuronal cells producing a second messenger response and expressing on their cell surface a human or rat Y4 receptor, with both the chemical compound and a second chemical compound known to activate the human or rat Y4 receptor, and with only the second chemical compound, under conditions suitable for activation of the human or rat Y4 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the human or rat Y4 receptor, wherein the human Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 2 and the rat Y4 receptor has an amino acid sequence as shown in SEQ ID NO: 28.

5. The process of claim 1 or 2, wherein the second messenger response comprises cAMP accumulation and the change in second messenger response is a reduction in cAMP accumulation.

6. The process of claim 3 or 4, wherein the second messenger response comprises cAMP accumulation and the change in second messenger response is a smaller reduction in cAMP accumulation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

7. The process of claim 1, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is an increase in intracellular calcium levels.

8. The process of claim 3, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is a smaller increase in the level of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

9. The process of any one of claims 1, 2, 3, or 4, wherein the cell is a mammalian cell.

10. The process of claim 9, wherein the mammalian cell is a COS-7 cell, a CHO cell, a LM(tk-) cell, a Y1 murine adrenal cell, or a NIH 3T3 cell.

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L2: Entry 7 of 7 File: USPT Feb 11, 1997

DOCUMENT-IDENTIFIER: US 5602024 A
**** See image for Certificate of Correction ****
TITLE: DNA encoding a·hypothalamic atypical neuropeptide Y/peptide YY receptor (Y5) and uses thereof

INVENTOR (1):
Gerald; Christophe P. G.

Brief Summary Text (6):
Rank orders of affinity for key peptides (NPY, PYY, PP, [Leu.sup.31, Pro.sup.34 ]NPY, NPY.sub.2-36, and NPY.sub.13-36) are based on previously reported binding and functional data (Schwartz et al., 1990; Wahlestedt et al., 1991; Dumont et al., 1992; Wahlestedt and Reis, 1993). Data for the Y2 receptor were disclosed in U.S. patent application Ser. No. 08/192,288 filed on Feb. 3, 1994, U.S. Pat. No. 5,545,549, the foregoing contents of which are hereby incorporated by reference. Data for the Y4 receptor were disclosed in U.S. patent application Ser. No. 08/176,412 filed on Dec. 28 1993, U.S. Pat. No. 5,516,653, the foregoing contents of which are hereby incorporated by reference. Missing peptides in the series reflect a lack of published information.

Brief Summary Text (9):
The Y1 receptor recognizes NPY.gtoreq.PYY>>PP (Grundemar et al., 1992). The receptor requires both the N- and the C-terminal regions of the peptides for optimal recognition. Exchange of Gln.sup.34 in NPY or PYY with the analogous residue from PP (Pro.sup.34), however, is well-tolerated. The Y1 receptor has been cloned from a variety of species including human, rat and mouse (Larhammar et al, 1992; Herzog et al, 1992; Eva et al, 1990; Eva et al, 1992). The Y2 receptor recognizes PYY.about.NPY>>PP and is relatively tolerant of N-terminal deletion (Grundemar et al., 1992). The receptor has a strict requirement for structure in the C-terminus (Arg.sup.33 -Gln.sup.34 -Arg.sup.35 -Tyr.sup.36 -NH.sub.2); exchange of Gln.sup.34 with Pro.sup.34, as in PP, is not well tolerated. The Y2 receptor has recently been cloned (disclosed in U.S. patent application Ser. No. 08/192,288, filed on Feb. 3, 1994 U.S. Pat. No. 5,545,549. The Y3 receptor is characterized by a strong preference for NPY over PYY and PP (Wahlestedt et al., 1991). [Pro.sup.34 ]NPY is reasonably well tolerated even though PP, which also contains Pro.sup.34, does not bind well to the Y3 receptor. This receptor (Y3) has not yet been cloned. The Y4 receptor (disclosed in U.S. patent application Ser. No. 08/176,412, filed on Dec. 28 1993 U.S. Pat. No. 5,576,653, binds PP>PYY>NPY. Like the Y1, the Y4 requires both the N- and the C-terminal regions of the peptides for optimal recognition (Synaptic Y4 patent). The "atypical Y1" or "feeding" receptor was defined exclusively by injection of several pancreatic polypeptide analogs into the paraventricular nucleus of the rat hypothalamus which stimulated feeding behavior with the following rank order: NPY.sub.2-36 .gtoreq.NPY.about.PYY.about.[Leu.sup.31, Pro.sup.34 ]NPY>NPY.sub.13-36 (Kalra et al., 1991; Stanley et al., 1992). The profile is similar to that of a Y1-like receptor except for the anomalous ability of NPY.sub.2-36 to stimulate food intake with potency equivalent or better than that of NPY. A subsequent report in J. Med. Chem. by Balasubramaniam and co-workers (1994) showed that feeding can be regulated by [D-Trp.sup.32 ]NPY. While this peptide was presented as an NPY antagonist, the published data at least in part support a stimulatory effect of [D-Trp.sup.32 ]NPY on feeding. [D-Trp.sup.32 ]NPY thereby represents another diagnostic tool for receptor identification. In contrast to other NPY receptor

subtypes, the "feeding" receptor has never been characterized for peptide binding affinity in radioligand binding assays and the fact that a single receptor could be responsible for the feeding response has been impossible to validate in the absence of an isolated receptor protein; the possibility exists, for example, that the feeding response could be a composite profile of Y1 and Y2 subtypes.

Detailed Description Text (109):
Human Y1, human Y2, and rat Y5 receptors were co-transfected with a G-418 resistant gene into the human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells were selected with G-418. Human Y4 receptors were similarly transfected into mouse fibroblast LMT(k)-cells.

Detailed Description Text (138):
The pharmacological profile of the rat Y5 receptor was first studied by using pancreatic polypeptide analogs in membrane binding assays. The rank order of affinity for selected compounds was derived from competitive displacement of .sup.125 I-PYY (FIG. 11). The rat Y5 receptor was compared with cloned Y1, Y2, and Y4 receptors from human (Table 4) and rat (Table 5), all expressed transiently in COS-7 cells. One receptor subtype absent from our panel was the Y3, human or rat, as no model suitable for radioligand screening has yet been identified.

Detailed Description Text (143):
The rat Y5 receptor possessed a unique pharmacological profile when compared with human and rat Y-type receptors. It displayed a preference for structural analogs of rat/human NPY (K.sub.i =0.68 nM) and rat/porcine PYY (K.sub.i =0.64 nM) over most PP derivatives. The high affinity for salmon PP (K.sub.i =0.31 nM) reflects the close similarity between salmon PP and rat NPY, sharing 81% of their amino acid sequence and maintaining identity at key positions: Tyr.sup.1, Gln.sup.34, and Tyr.sup.36. Both N- and C-terminal peptide domains are apparently important for receptor recognition. The N-terminal tyrosine of NPY or PYY could be deleted without an appreciable loss in binding affinity (K.sub.i =0.86 nM for rat/human NPY.sub.2-36), but further N-terminal deletion was disruptive (K.sub.i =73 nM for porcine NPY.sub.13-36). This pattern places the binding profile of the Y5 receptor somewhere between that of the Y2 receptor (which receptor can withstand extreme N-terminal deletion) and that of the Y1 receptor (which receptor is sensitive to even a single-residue N-terminal deletion). Note that the human Y4 receptor can be described similarly (K.sub.i =0.06 nM for human PP, 0.06 nM for human PP.sub.2-36, and 39 nM for human PP.sub.13-36). The Y5 receptor resembled both Y1 and Y4 receptors in its tolerance for ligands containing Pro.sup.34 (as in human [Leu.sup.31,Pro.sup.34 ]NPY, human [Pro.sup.34 ]-PYY, and human PP). Interestingly, the rat Y5 receptor displayed a preference for human PP (K.sub.i =5.0 nM) over rat PP (K.sub.i =180 nM). This pattern distinguishes the rat Y5 from the rat Y4 receptor, which binds both human and rat PP with K.sub.i values <0.2 nM. Hydrolysis of the carboxy terminal amide to free carboxylic acid, as in NPY free acid, was disruptive for binding affinity for the rat Y5 receptor (K.sub.i =480 nM). The terminal amide appears to be a common structural requirement for pancreatic polypeptide family/receptor interactions.

Detailed Description Text (154):
Binding data were generated as described in Tables 4 and 5. Functional data were derived from radioimmunoassay of cAMP accumulation in stably transfected cells stimulated with 10 .mu.M forskolin. [D-Trp.sup.32 ]NPY was tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 .mu.M. Alternatively, [D-Trp.sup.32 ]NPY was included as a single spike (0.3 .mu.M) in the human PYY concentration curve for human Y1 and human Y2 receptors, or in the human PP concentration curve for human Y4 receptors, and antagonist activity was detected by the presence of a rightward shift (from EC.sub.50 to EC.sub.50 '). K.sub.b values were calculated according to the equation: K.sub.b =[[D-Trp.sup.32 ]NPY/((EC.sub.50 /EC.sub.50 ')-1). The data shown are representative of at least two independent experiments.

Detailed Description Text (162):
The nucleotide and amino acid sequence analysis of Y5 (rat and human) reveals low identity levels with all 7 TM receptors including the Y1, Y2 and Y4 receptors, even in the transmembrane domains which are usually highly conserved within receptor subfamilies. Applicants have named CG-18 and CG-19 "Y5" receptors because of their unique amino acid sequence (87.2% identical with each other, .ltoreq.42% identical

with the TM regions of previously cloned "Y" receptor subtypes) and pharmacological profile. The name is not biased toward any one member of the pancreatic polypeptide family. The "Y" has its roots in the original classification of Y1 and Y2 receptor subtypes (Wahlestedt et al., 1987). The letter reflects the conservation in pancreatic polypeptide family members of the C-terminal tyrosine, described as "Y" in the single letter amino acid code. The number is the next available in the Y-type series, position number three having been reserved for the pharmacologically defined Y3 receptor. Applicants note that the cloned human Y1 receptor was introduced by Larhammar and co-workers as a "human neuropeptide Y/peptide YY receptor of the Y1 type" (Larhammar et al., 1992). Similarly, the novel clones described herein can be described as rat and human neuropeptide Y/peptide YY receptors of the Y5 type. The rat hypothalamic Y5 receptor displays a very similar pharmacological profile to the pharmacologically described "atypical" Y1 receptor thought to mediate NPY-induced food intake in rat hypothalamus. Both the Y5 receptor and the "feeding receptor" display a preference for NPY and PYY-like analogs, a sensitivity to N-terminal peptide deletion, and a tolerance for Pro.sup.34. Each would be considered Y1-like except for the anomalous ability of NPY.sub.2-36 to bind and activate as well as NPY. Each appears to be sensitive to changes in the mid-region of the peptide ligand. For example, a study by Kalra and colleagues (1991) indicated that replacement of the NPY midregion by an amino-octanoic chain to produce NPY.sub.1-4 -Aca-.sub.25-36 dramatically reduced activity in a feeding behavioral assay. Likewise, applicants note that the robust difference in human PP binding (K.sub.i =5.0 nM) and rat PP binding (K.sub.i =230) to the rat Y5 receptor can be attributed to a series of 8 amino acid changes between residues 6-30 in the peptide ligands, with human PP bearing the closer resemblance to human NPY. These matching profiles, combined with a selective activation of the rat Y5 by the reported feeding "modulator" [D-Trp.sup.32 ]NPY, support the identity of the rat Y5 as the "feeding receptor" first proposed to explain NPY-induced feeding in rat hypothalamus.

Detailed Description Text (165):
The Y5 pharmacological profile offers a new standard by which to review the molecular basis of all NPY-dependent processes; examples are listed in Table 11. Such an exercise suggests that the Y5 receptor is likely to have a physiological significance beyond feeding behavior. It has been reported, for example, that a Y-type receptor can regulate luteinizing hormone releasing hormone (LHRH) release from the median eminence of steroid-primed rats in vitro with an atypical Y1 pharmacological profile. NPY, NPY.sub.2-36, and LP-NPY were all effective at 1 uM but deletion of as few as four amino acids from the N-terminus of NPY destroyed biological activity. The Y5 may therefore represent a therapeutic target for sexual or reproductive disorders. Preliminary in situ hybridization of rat Y5 mRNA in hippocampus and elsewhere further suggest that additional roles will be uncovered, for example, in the regulation of memory. It is worth while considering that the Y5 is so similar in pharmacological profile to the other Y-type receptors that it may have been overlooked among a mixed population of Y1, Y2 and Y4 receptors. Certain functions now associated with these subtypes could therefore be reassigned to Y5 as our pharmacological tools grow more sophisticated (Table 12). By offering new insight into NPY receptor pharmacology, the Y5 thereby provides a greater clarity and focus in the field of drug design.